Expanded View Figures

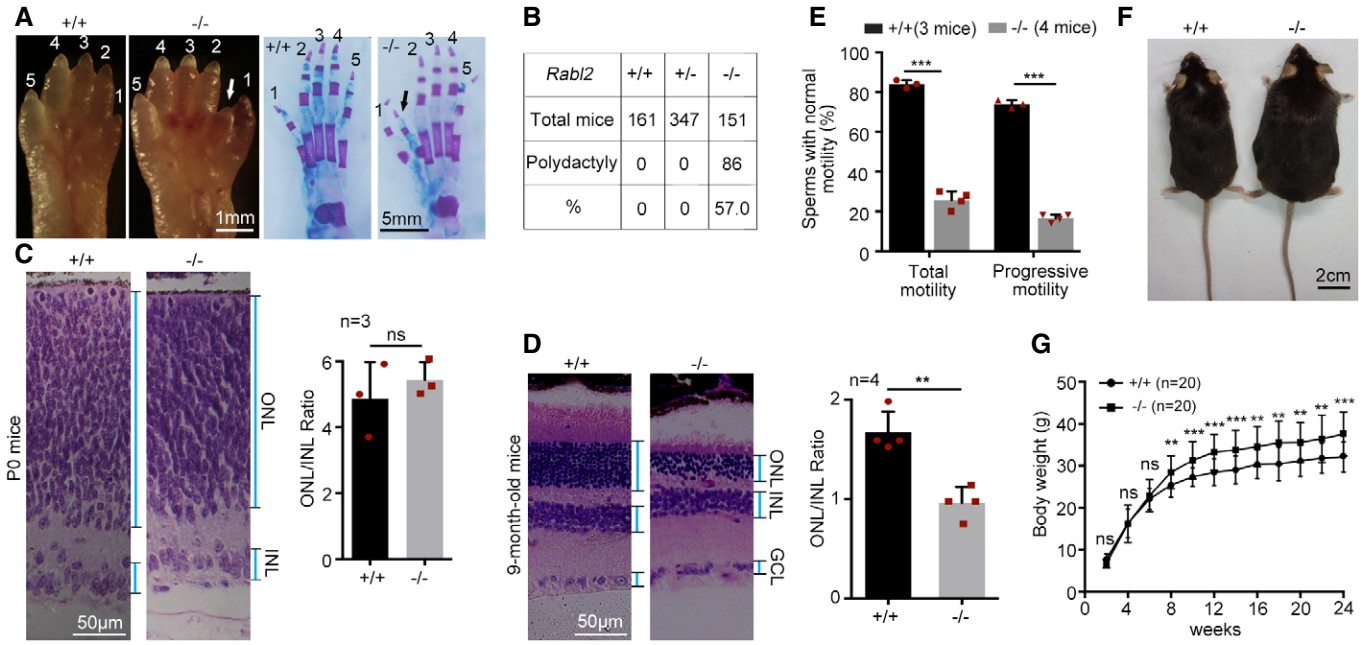

Figure EV1. *Rabl2* null mice display ciliopathy-like phenotypes (related to Fig 1).

A, B *Rabl2*$^{-/-}$ neonatal mice manifested polydactyly. Unstained (left) and alcian blue-stained (right) hind limbs (A) reveal preaxial duplication of digit 1 (arrow) in hind limbs of *Rabl2*$^{-/-}$ P0 mice. Mice with polydactyly at one (n = 62) or both sides (n = 24) of hind limbs were scored (B).

C, D *Rabl2*$^{-/-}$ mice suffered from retina degeneration. Typical H&E stained retina sections from P0 (C) or 9-month-old (D) littermates are presented. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

E *Rabl2*$^{-/-}$ male mice produced sperms with defective motility. Sperms from 2-month-old male mice were used for motility assays.

F, G Male *Rabl2*$^{-/-}$ mice were obese as compared to their wildtype littermates. A typical pair of 6-month-old littermates are shown in (F). Growth curves are shown in (G).

Data information: Pooled data are presented as mean ± s.d. Student's t-test: ns, no significance; $^{**}P < 0.01$; $^{***}P < 0.001$.

Source data are available online for this figure.

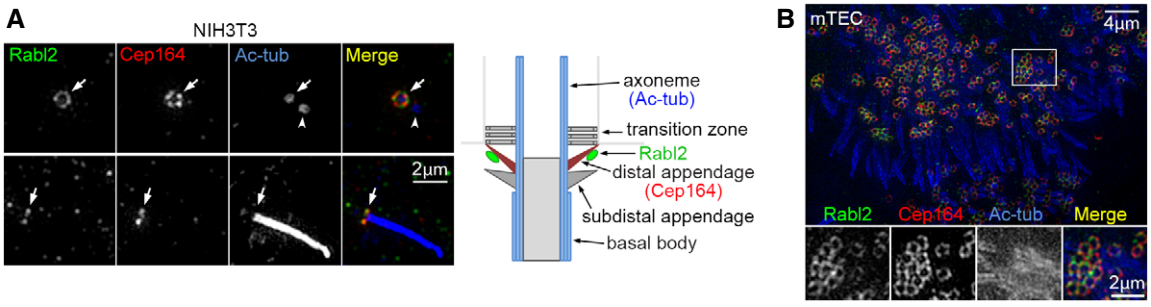

Figure EV2. Rabl2 is enriched at distal appendages in both primary and motile cilia (related to Fig 1).

A Super-resolution images of primary cilia. NIH3T3 cells serum-starved for 48 h to induce primary cilia were immunostained and imaged through Three Dimensional Structured Illumination Microscopy (3D-SIM). Cep164 served as marker for distal appendages (or transition fibers) (Yang *et al*, 2015). Acetylated tubulin (Ac-tub) labeled the axoneme (bottom panel) or centrioles (top panel). The arrows and arrowheads indicate positions of mother centrioles and the daughter centriole, respectively. The diagram illustrates ciliary structure and localization of Rabl2.

B 3D-SIM images of motile multicilia. mTECs were cultured at an air-liquid interface (ALI) to induce multicilia formation. The framed region was magnified to show details.

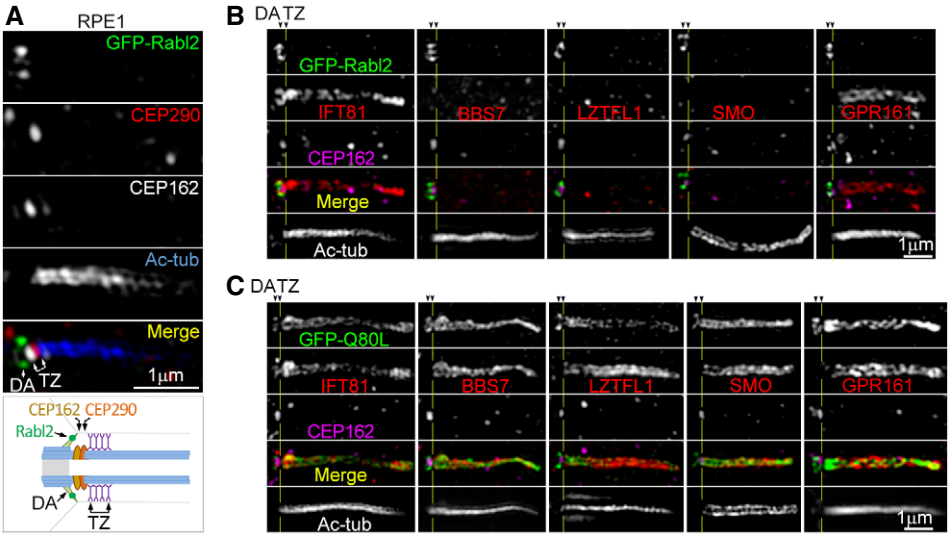

Figure EV3. Rabl2^{Q80L} induces ciliary accumulation of BBS proteins and GPCRs above TZ (related to Fig 5).

A 3D-SIM images showing the spatial relationship among different makers in a presentative primary cilium of RPE1 cells. Transition zone (TZ) markers CEP290 and CEP162 were closely spaced at the base of Ac-tub-labeled axoneme. GFP-Rabl2 localized to distal appendages (DA), similar to endogenous Rabl2 (see Fig EV2A). The diagram illustrates their locations relative to TZ (Yang *et al*, 2015; Garcia-Gonzalo & Reiter, 2017).

B, C Distribution of the indicated proteins (red) in cilia. RPE1 cells expressing GFP-Rabl2 (B) or GFP-Rabl2^{Q80L} (C) were imaged through 3D-SIM. Yellow lines mark the middle of TZ.

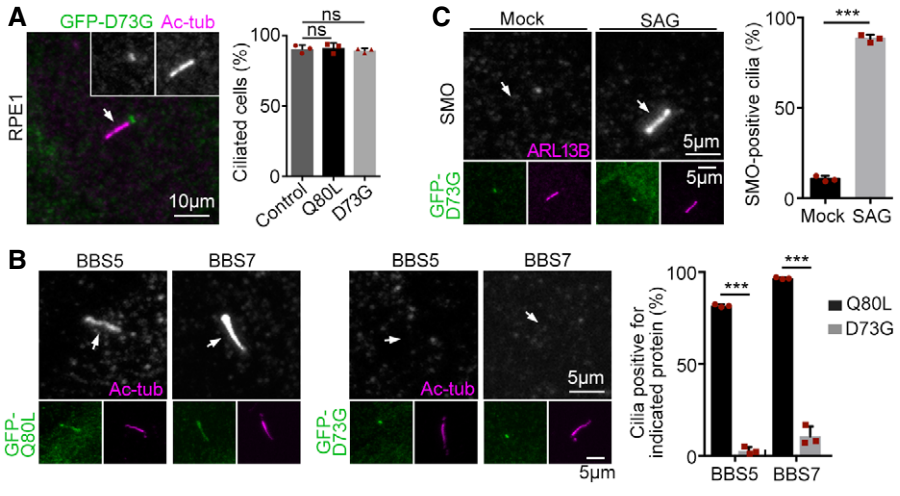

Figure EV4. Rabl2^{D73G} does not repress ciliogenesis and ciliary exit of BBSome and SMO (related to Fig 6).

A Rabl2^{D73G} did not affect ciliogenesis efficiency. RPE1 cells that were either uninfected (control) or infected with adenovirus to express GFP-tagged Rabl2^{Q80L} or Rabl2^{D73G} were serum-starved for 48 h. The white arrow denotes cilium. At least 100 cells were scored in each experiment and condition.

B Rabl2^{D73G} did not cause ciliary BBSome accumulation. Rabl2^{Q80L} served as positive control. The white arrows indicate positions of cilia. At least 105 cells were scored in each experiment and condition.

C Rabl2^{D73G} did not alter ciliary translocation of SMO. RPE1 cells expressing GFP-Rabl2^{D73G} were serum-starved for 24 h and treated with DMSO (mock) or SAG for an additional 24 h prior to immunostaining. The white arrows indicate positions of cilia. At least 100 cells were scored in each experiment and condition.

Data information: Quantification results are presented as mean ± s.d. Student's *t*-test: ns, no significance; ***$P < 0.001$.

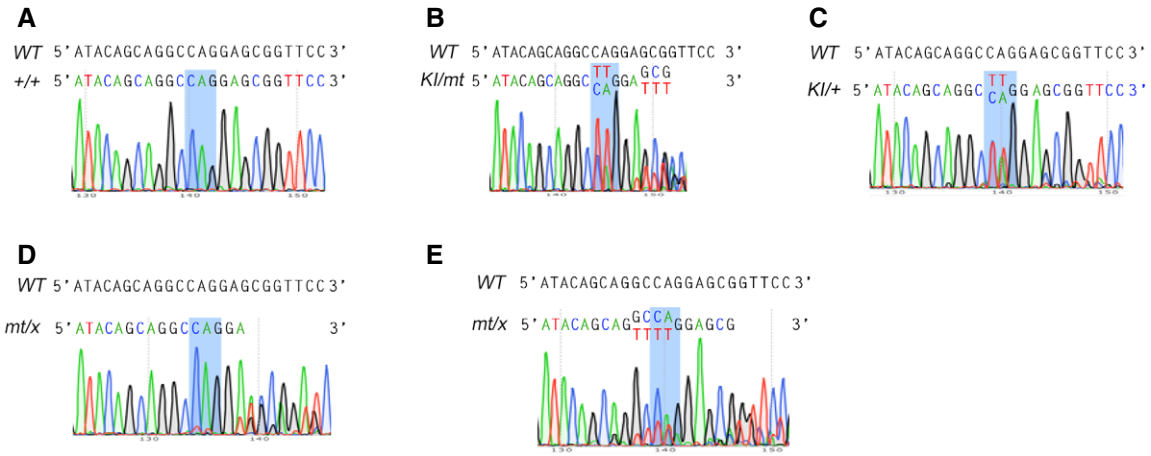

Figure EV5. Additional representative genotyping results for *Rabl2* gene-edited mice (related to Fig 7).

A–E Representative sequencing results for the *Rabl2*$^{+/+}$ (A) and putatively *Rabl2*$^{KI/mt}$ (B), *Rabl2*$^{KI/+}$ (C), and *Rabl2*$^{mt/x}$ (D, E) groups of mice. The *Rabl2* fragments covering the expected Cas9-cleavage region were amplified by PCR from tail genomic DNAs prepared, respectively, from *Rabl2* gene-edited neonatal mice (Fig 7A) and subjected to sequencing analyses. The blue boxes mark the position of the codon (CAG) for glutamine-80 (Q^{80}) in wildtype (WT) *Rabl2*. 61 mice showed sequencing results of mixed peaks after the CAG codon (D), whose genotypes are putatively *Rabl2*$^{mt/+}$ or *Rabl2*$^{mt/mt}$. 7 mice showed mixed peaks before the CAG codon (E) and are putatively *Rabl2*$^{mt/+}$, *Rabl2*$^{mt/mt}$, or *Rabl2*$^{mt/KI}$. We thus used *x* to represent the ambiguous allele. As some of these "heterozygous" mice might actually be chimeric ones, the putative genotypes are used only for simplicity.

Source data are available online for this figure.